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Brief Technical Note

HATCHING EVENTS IN THE CYSTS OF *ARTEMIA SALINA*

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ABSTRACT

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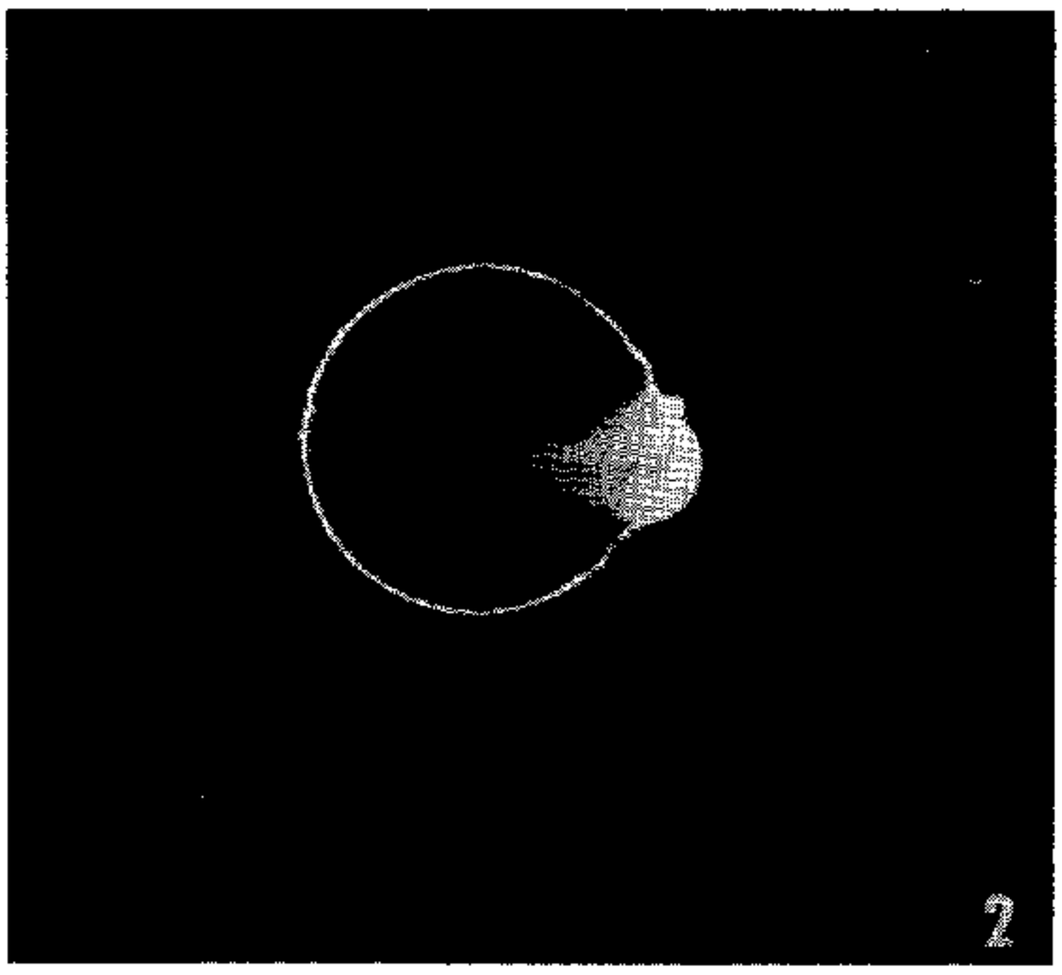
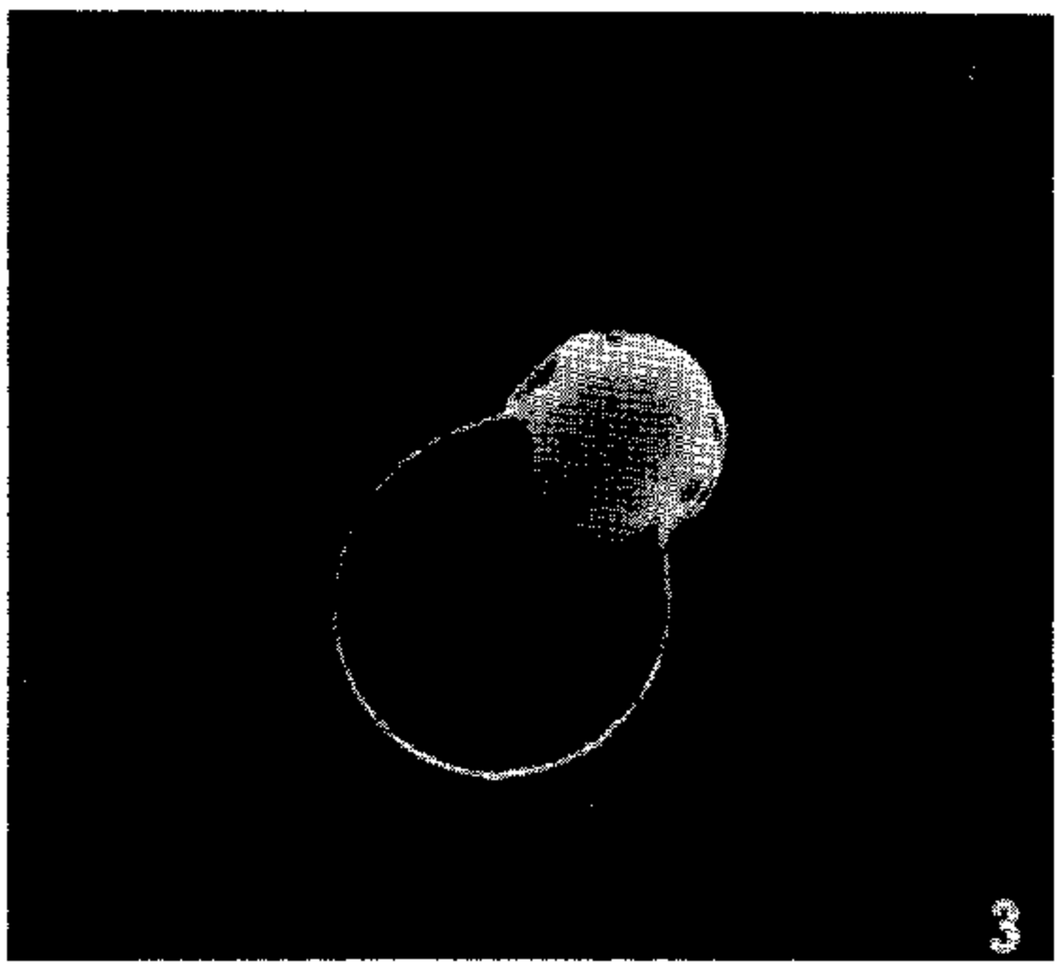
Prior to hydration, the cysts of *Artemia salina* are cup-shaped with a diameter of approximately 0.18 mm. Upon immersion in sea water, the cysts slightly increase in diameter to 0.19 mm and assume a spherical shape. Hatching begins with the splitting of the surface coat. The split runs along a straight line, approximately one-half the circumference of the cyst. The exposed fracture in the surface coat reveals three distinct regions: (1) a thin (1.2 μm) smooth outer layer; (2) a thick (4.7 μm) 'spongy' layer; and (3) a thin (1.8 μm) fibrous inner layer. The split becomes more pronounced as the nauplius, enclosed within a transparent hatching membrane, slowly emerges. Once completely emerged from the cyst, the nauplius begins a series of beating movements which rupture the hatching membrane, allowing the nauplius to swim free.

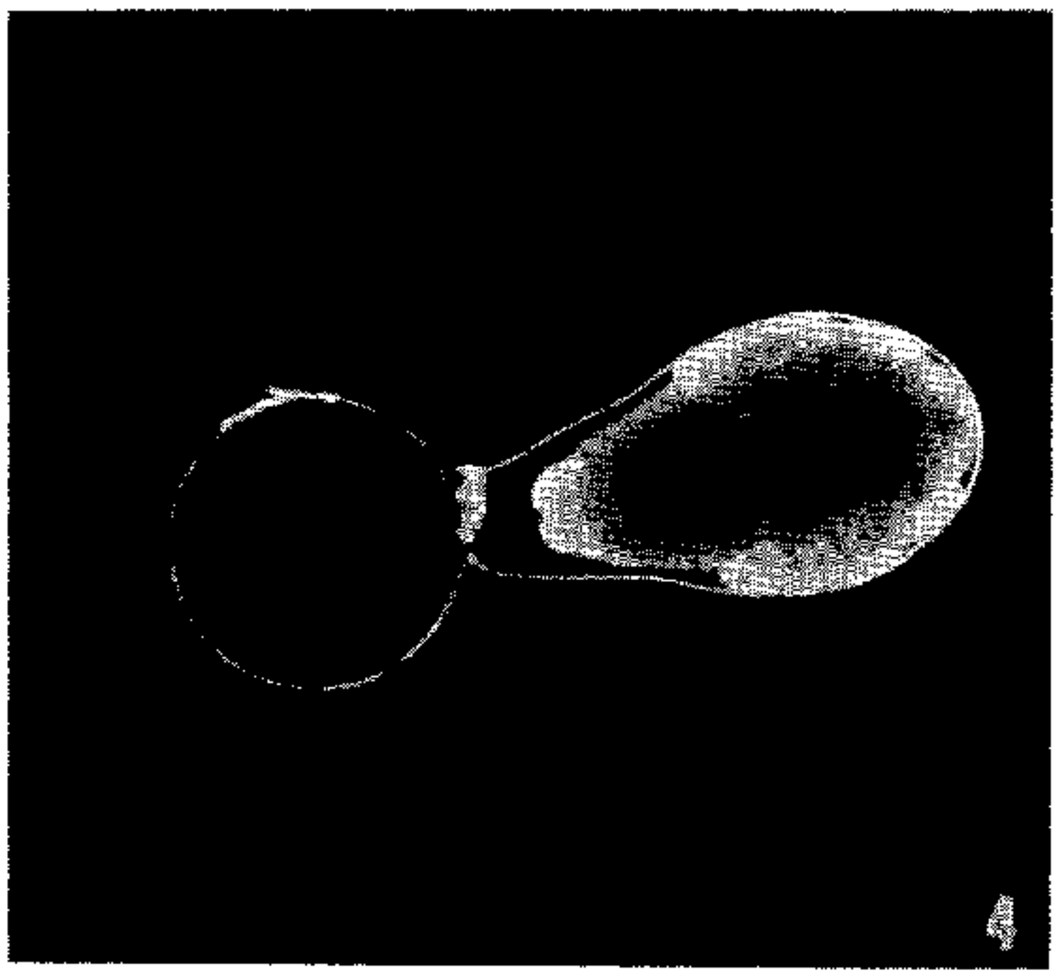
INTRODUCTION

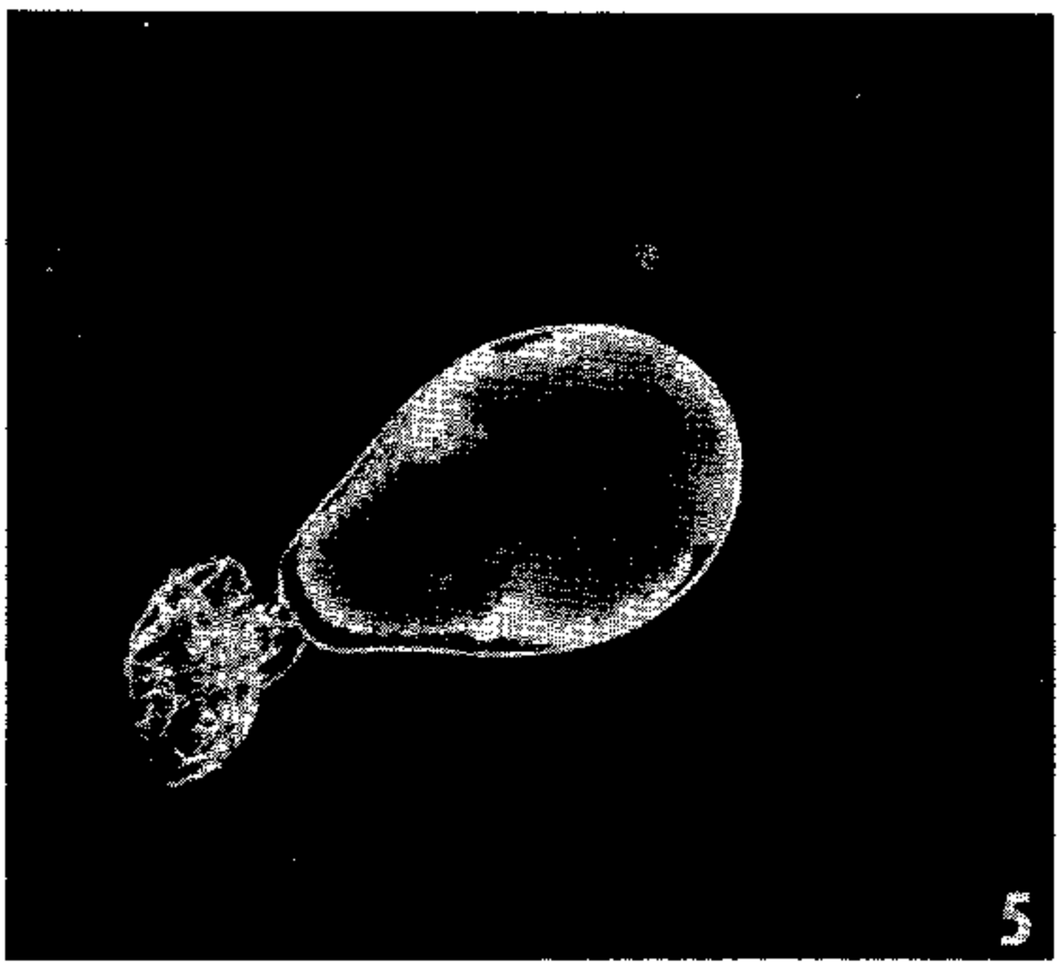
Brine shrimp have fascinated investigators for years with their unusual physiological features. One such unique characteristic is their capability of developing both eggs and cysts. Female brine shrimp produce a brood of eggs about every 4 days. Each brood will develop either a thin or thick protective shell. Thin-shelled eggs hatch within a few days and are released ovoviporously while thick-shelled eggs stop development at gastrulae and are released as cysts.

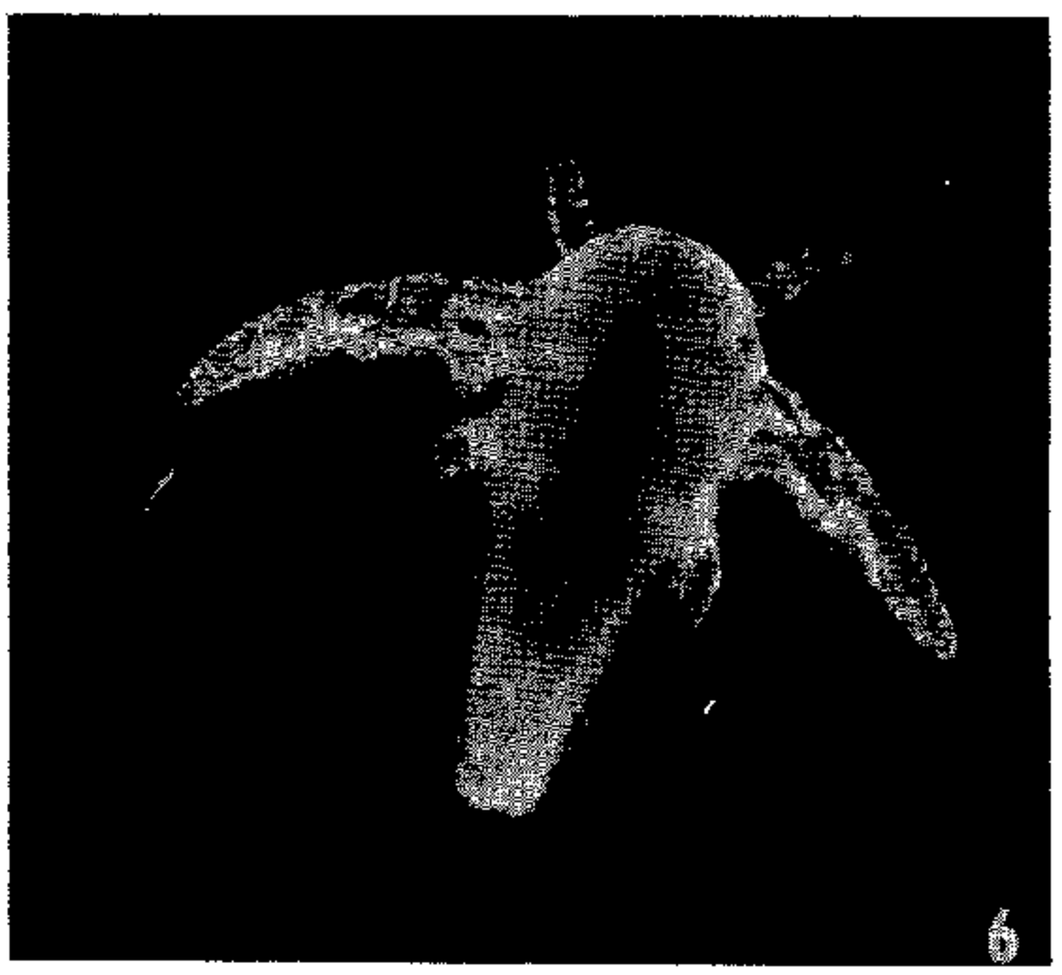
The cysts are able to withstand prolonged periods of desiccation. The capability of storing artemia cysts for long time periods, coupled with the animals' relative hardiness and high nutritional value as a feed for numerous aquatic animals, has made brine shrimp extremely valuable for aquaculture. Since the value of cysts is unquestioned, problems associated with their procurement and use are of the utmost importance. Artemia cysts have become so costly that they often represent a large economic consideration in terms of facility operation. Thus, cysts exhibiting poor hatchability are of considerable consternation. In other instances cysts have been suspected of being transmitters of disease organisms.











Figs. 1—6. Dark-field microscopic examination of the hatching process (× 70).

We have initiated studies, utilizing the scanning electron microscope, to examine the hatching events and larval development of *Artemia*. This paper presents the first phase of this study, which may be helpful to aquaculturists in understanding better the hatching process and problems related to bacterial fouling of brine shrimp cysts.

MATERIAL AND METHODS

San Francisco Bay brand cysts were placed in constantly aerated salt water with a salinity of $32^0/_{00}$ and a temperature of 28°C. For light microscopic studies, samples were taken between the 16th and 20th hour post-hydration and fixed in seawater buffered 2% glutaraldehyde. The various stages were photographed with dark-field optics. Specimens prepared for scanning electron microscope were fixed in seawater buffered 1% osmium tetroxide for 1—3 h, dehydrated in a graded acetone series and critical point dried in a Samdri PVT-3. The specimens were coated with 100 Å of both carbon and gold and viewed on a Cambridge Stereoscan 410.

RESULTS AND DISCUSSION

Light microscopy

The cysts assume a spherical shape 1—2 h after introduction to sea water (Fig.1). Samples taken between the 16th and 20th hour reveal cysts in various stages of hatching. Hatching begins with the splitting of the shell. The nauplius, surrounded by a hatching membrane, slowly emerges from the ruptured cyst (Figs. 2—3). The process continues until the nauplius is completely out but still attached to the cyst (Fig. 4). The 'pear-shaped' encased nauplius is attached to the shell by the inner cuticular membrane which is clearly demonstrated in Fig. 5. The nauplius begins a series of intermittent beatings until the hatching membrane breaks, allowing the nauplius to swim free (Fig. 6).

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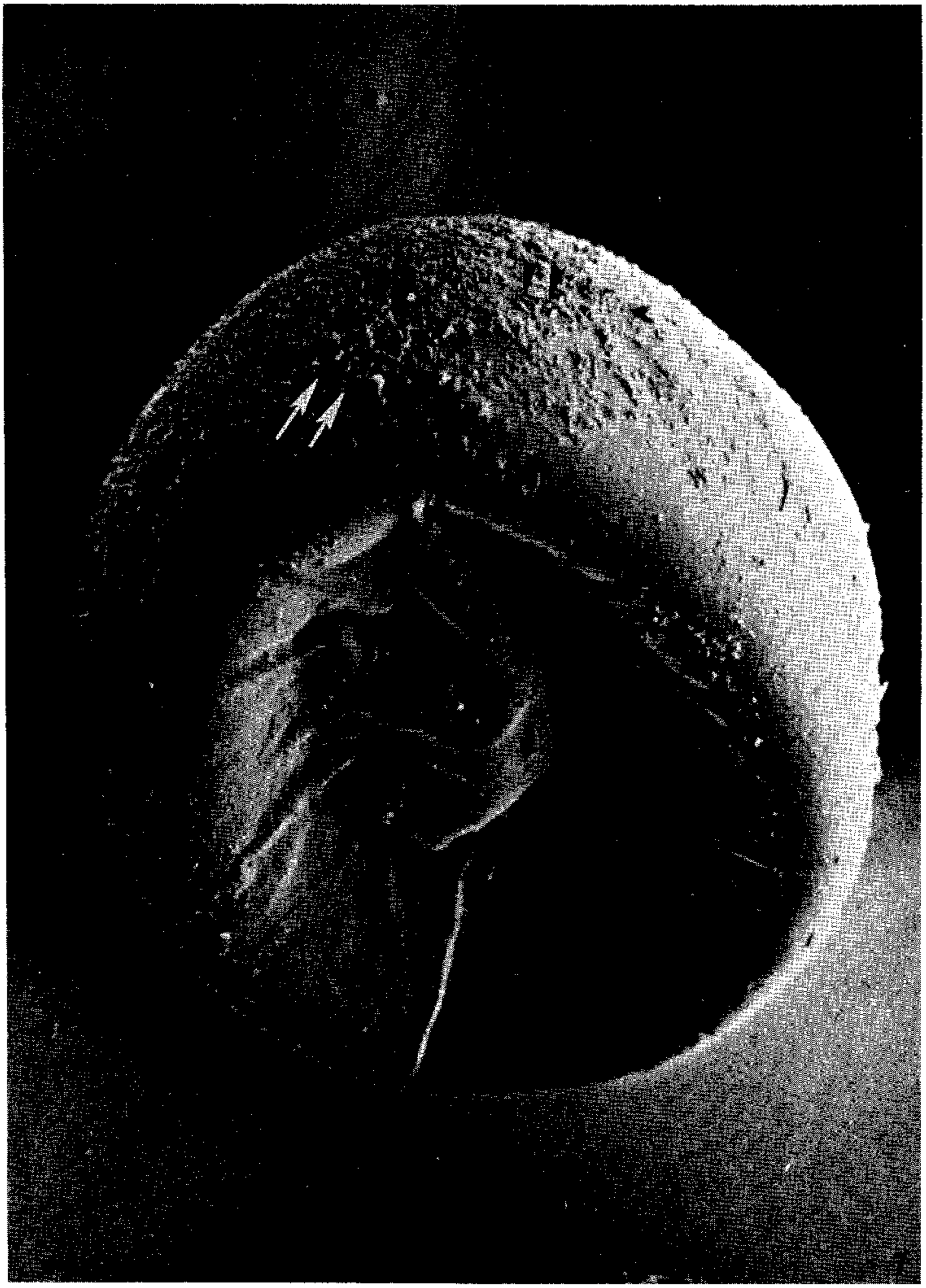
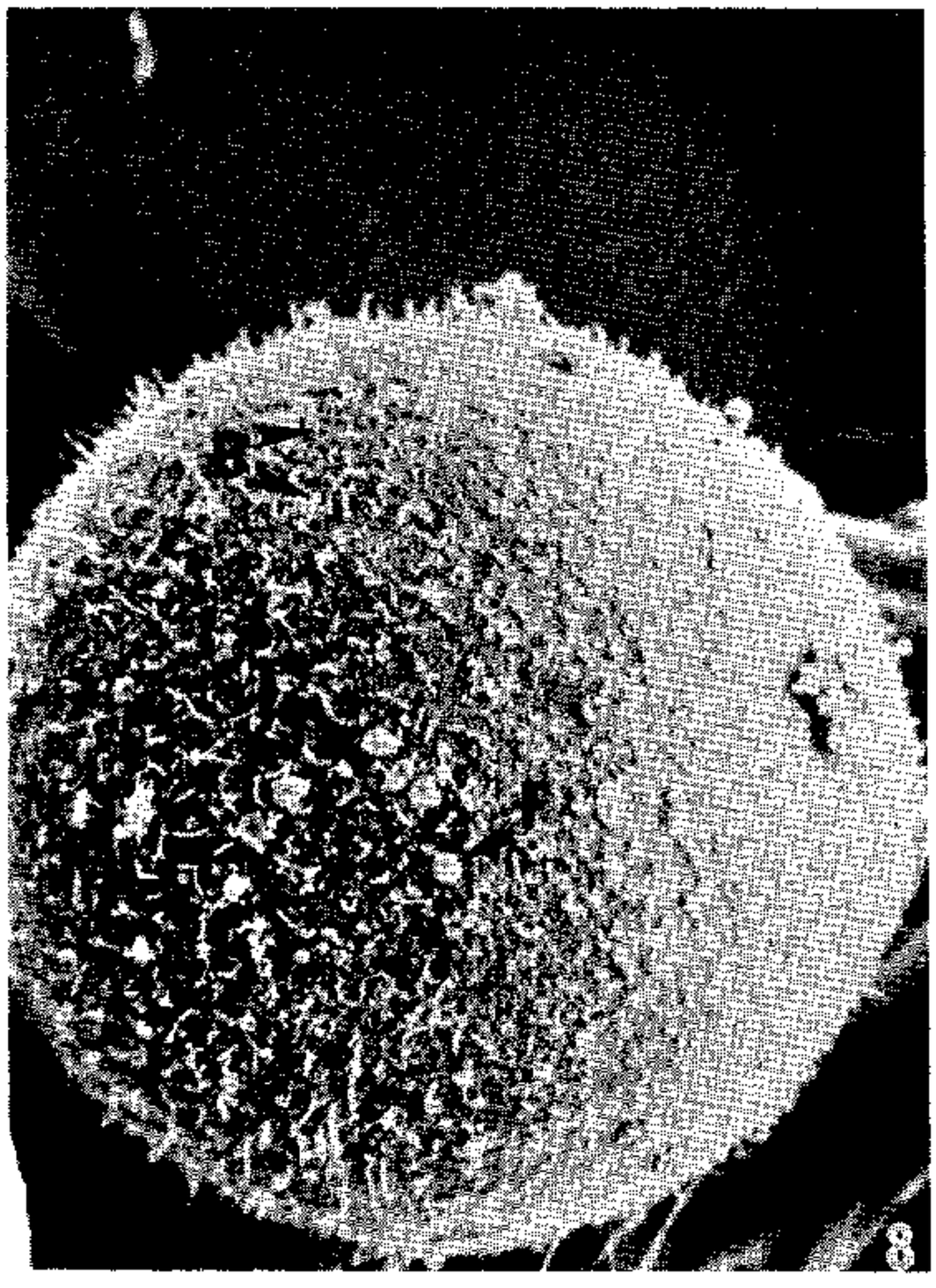


Fig.7. A scanning electron micrograph of a dehydrated cyst. Note the one large indentation as well as the microbial material (arrows) covering the outer surface coat of the cyst shell (× 560).



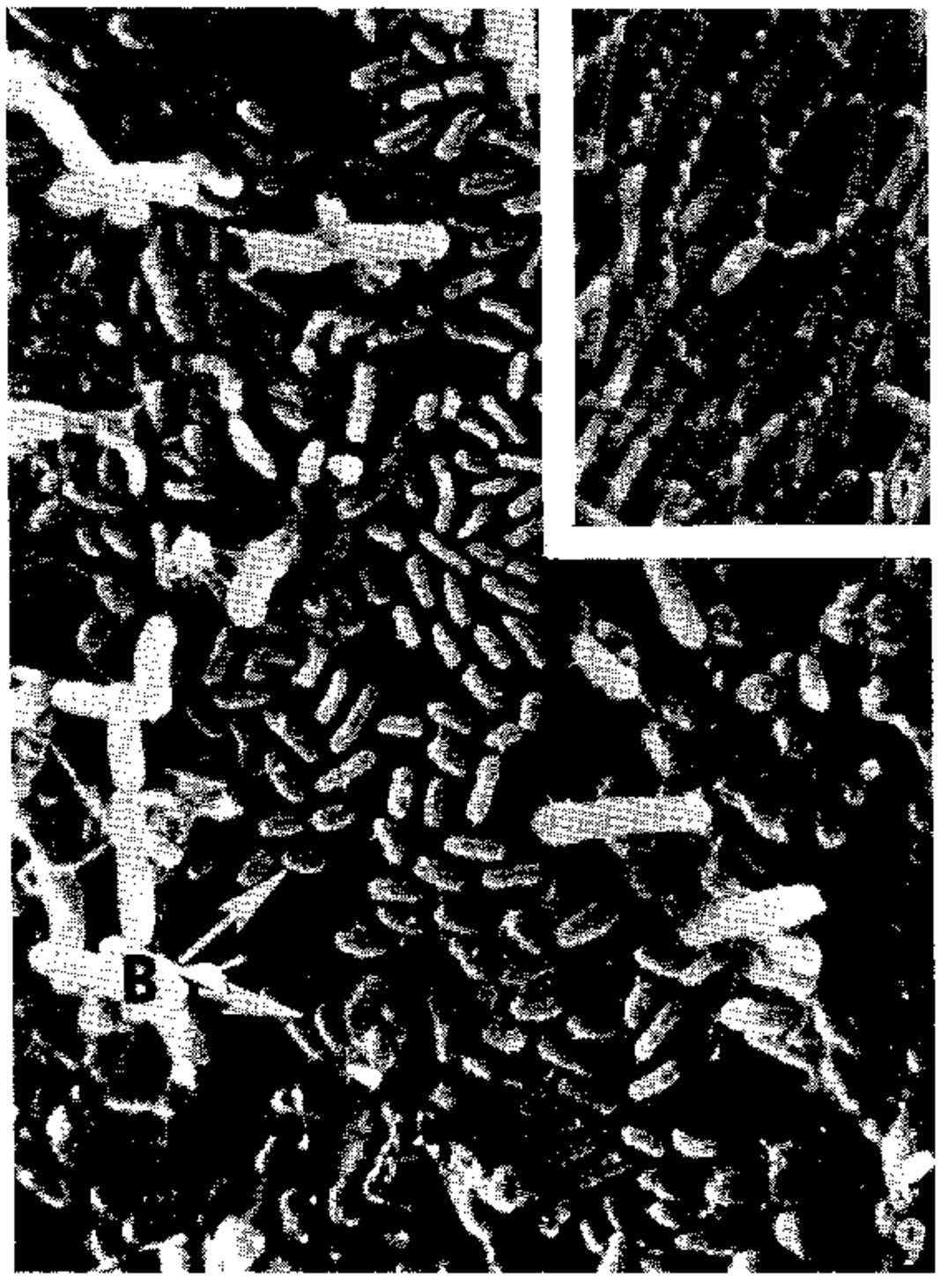


Fig.8. A cyst which has been hydrated. Note the bacterial (B) as well as possible fungal (F) infection on the surface (× 260).

Fig.9. A higher magnification showing the bacteria (B) attached to, as well as embedded within, the outer layer of the shell (× 2965).

Fig.10. A high magnification of a cyst, which had not hatched after 22 h. The prolific population of microbes blocks the view of the shell (× 3250).

Scanning microscopy

Before hydration the cysts resemble a deflated ball (Fig. 7). Within 2 h the deep indentation is lost and the cysts assume a spherical shape (Fig. 8). This transformation is the result of rapid absorption of water which approximately doubles the weight of the cyst (Clegg, 1964). The size of the cyst varies from an average of 0.178 mm in diameter prior to hydration to approximately 0.190 after 2 h in salt water.

Bacteria are commonly found associated with the shell surface of most cysts. The degree of contamination varies considerably, but often bacteria are found completely coating the outer shell layer (Fig. 9). Cysts that are unhatched after 22—24 h reveal such a heavy bacterial flora that the outer shell layer is completely encrusted (Fig. 10). Bacterial infections of *Artemia* cysts and their possible effect on cultured organisms have been previously reported (Shelbourne, 1964; Gilmour et al., 1975). The use of decapsulation as described by Sorgeloos et al. (1977) may decrease the possibility of transferring bacterial infections and possibly result in greater hatching percentages (Morris and Afzelius, 1967).

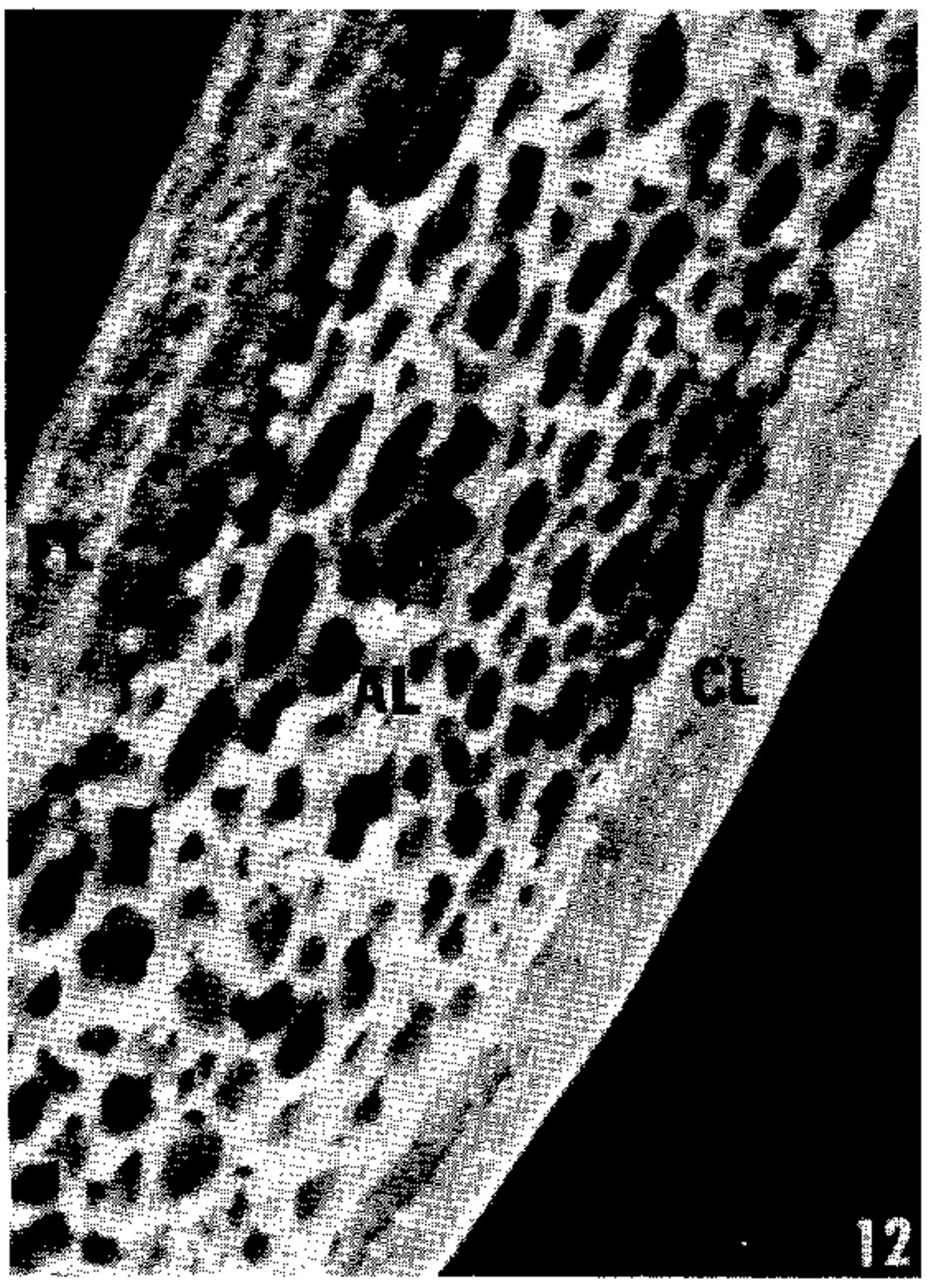
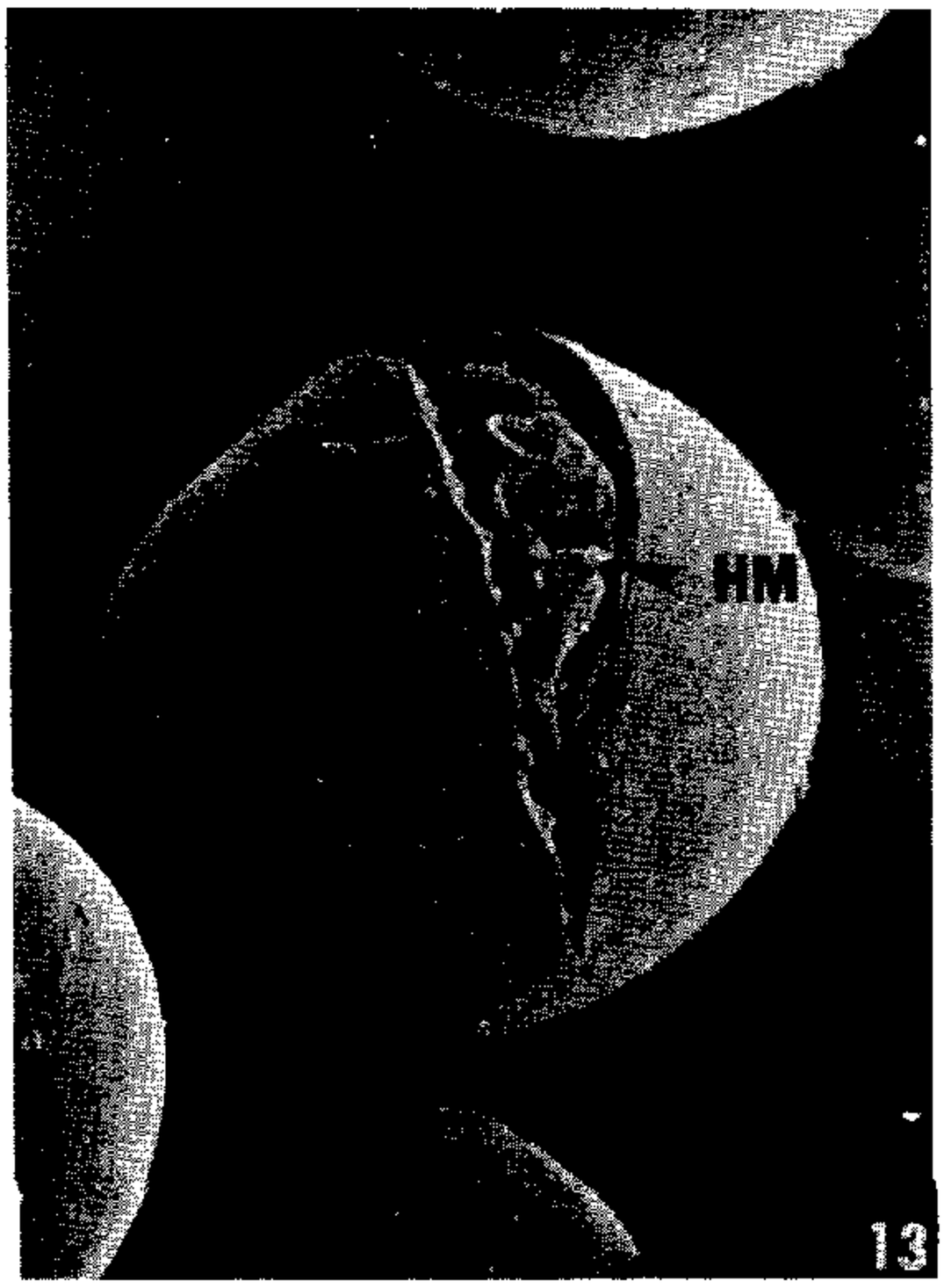


Fig.11. When hatching is initiated, the shell ruptures exposing its three distinct regions (arrows) (× 530).

Fig.12. A cross-section of the cyst's shell. Cortical layer CL, alveolar layer AL, fibrous layer FL (× 5798).



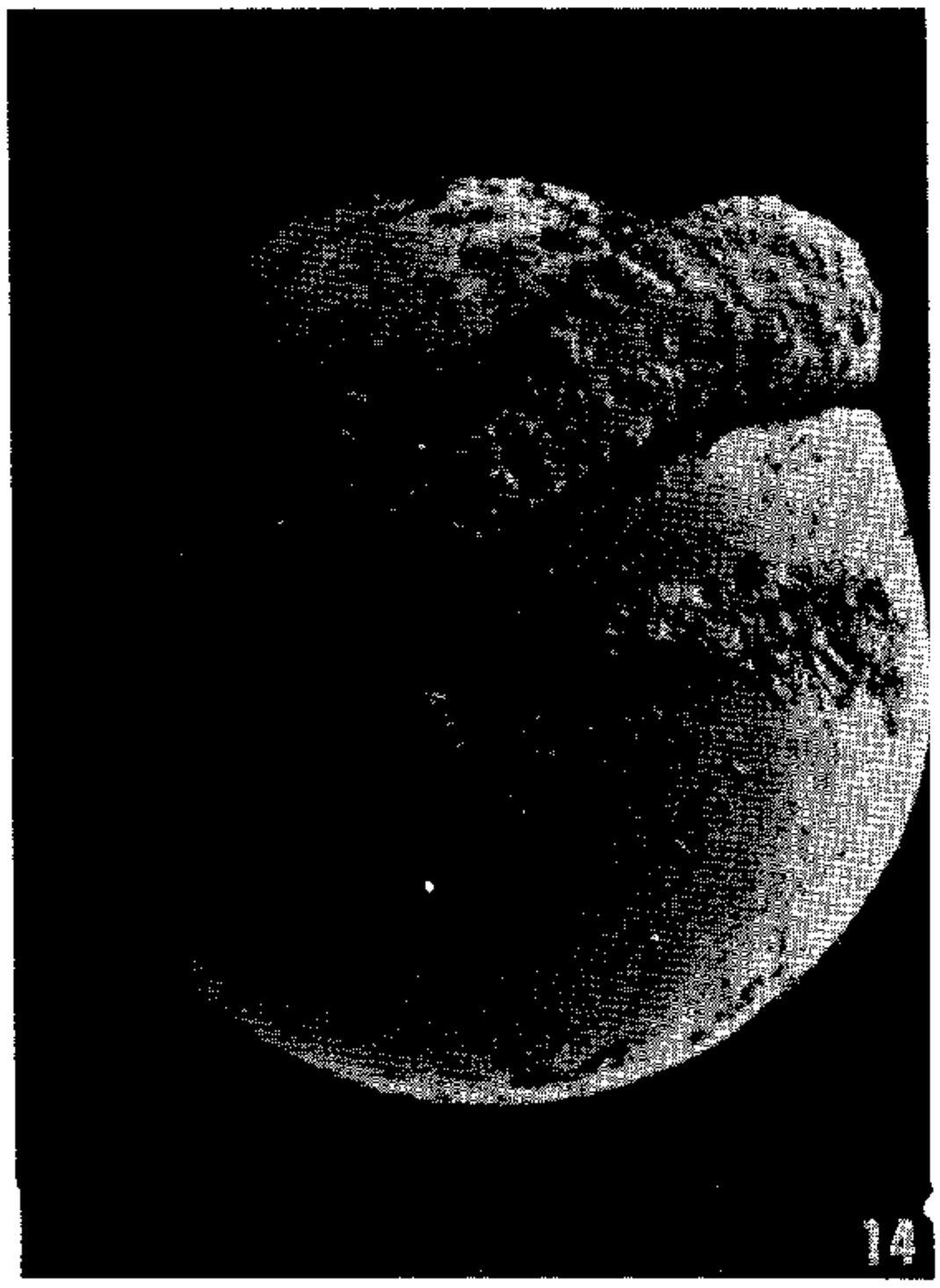


Fig.13. The split widens to accommodate the emerging nauplius. The nauplius is sheathed in a thin hatching membrane (HM) (× 222).

Fig.14. The nauplius begins to flow out, resembling a thick viscous fluid (× 286).

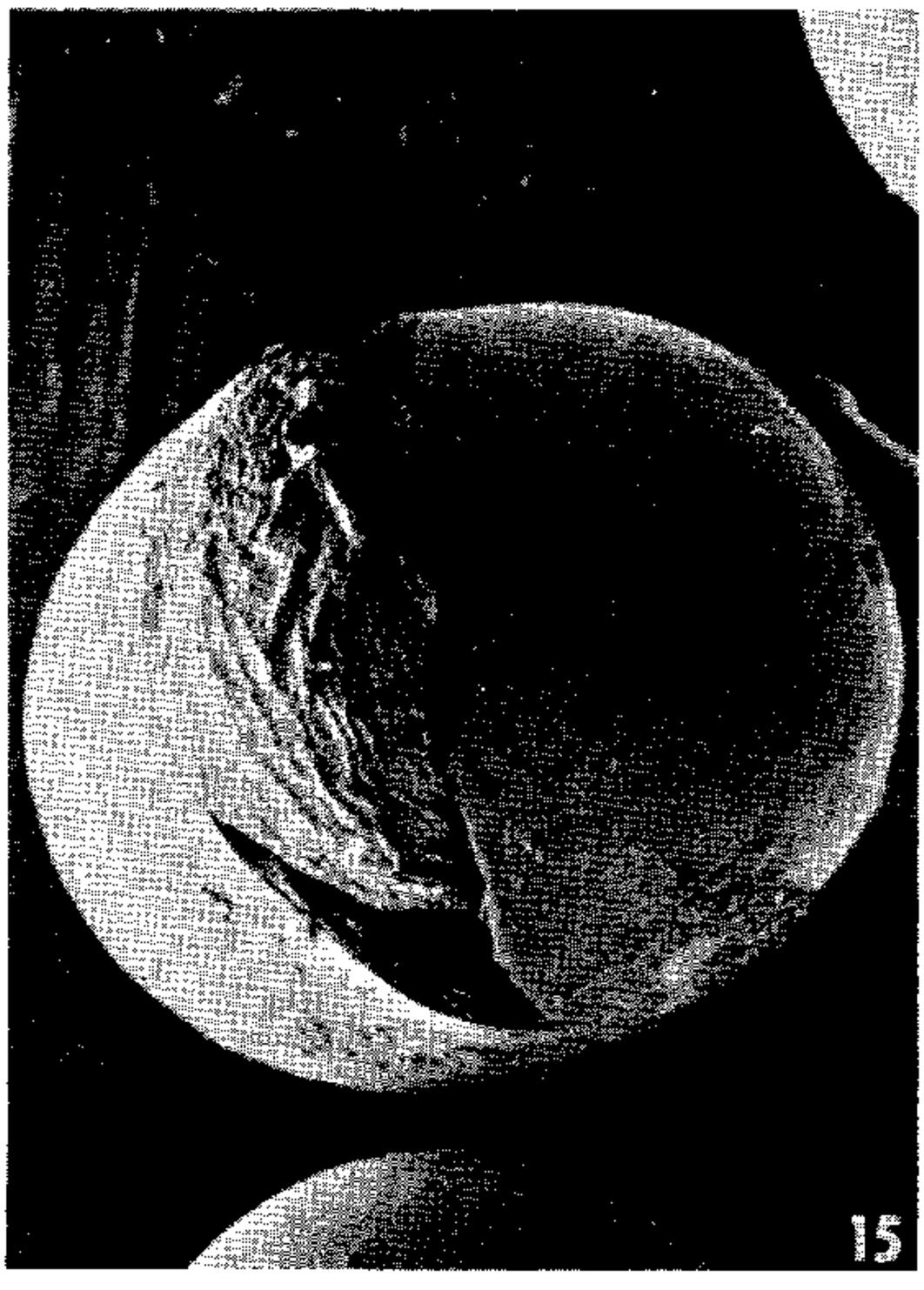


Fig.15. As the split enlarges, the nauplius continues to emerge (× 254).

Fig.16. Once completely emerged from the cyst, the nauplius remains attached to the shell by the inner cuticular membrane (ICM) (× 165).

Fig.17. A slightly higher magnification showing the attachment (× 260).

When the nauplius is fully developed, the shell splits, which is the result of free glycerol production and causes a considerable uptake of water (Clegg, 1964). The complex nature of the shell is revealed in partially-ruptured cysts with the scanning electron microscope. Three distinct regions are clearly discernable (Figs 11—12). These results correspond with the transmission electron microscopic studies of Morris and Afzelius (1967) and Anderson et al. (1970). The outermost region of the shell is smooth in texture, 1.2 μm in thickness and consists of the outer membrane and cortical layer. The middle region appears spongy in morphology; it is 4.7 μm in width and corresponds with the alveolar layer, and the inner region is fibrous in appearance, 1.8 μm in width and is composed of the outer cuticular membrane, fibrous layer, and inner cuticular membrane.

Once the shell ruptures, the nauplius slowly emerges (Figs 13—15). The extruded larva is ensheathed in a hatching membrane (Fig. 16) which remains attached to the shell, apparently by the inner cuticular membrane (Fig. 17). Shortly after extrusion, the nauplius begins a series of beating movements. These movements result in the rupturing of the hatching mem-

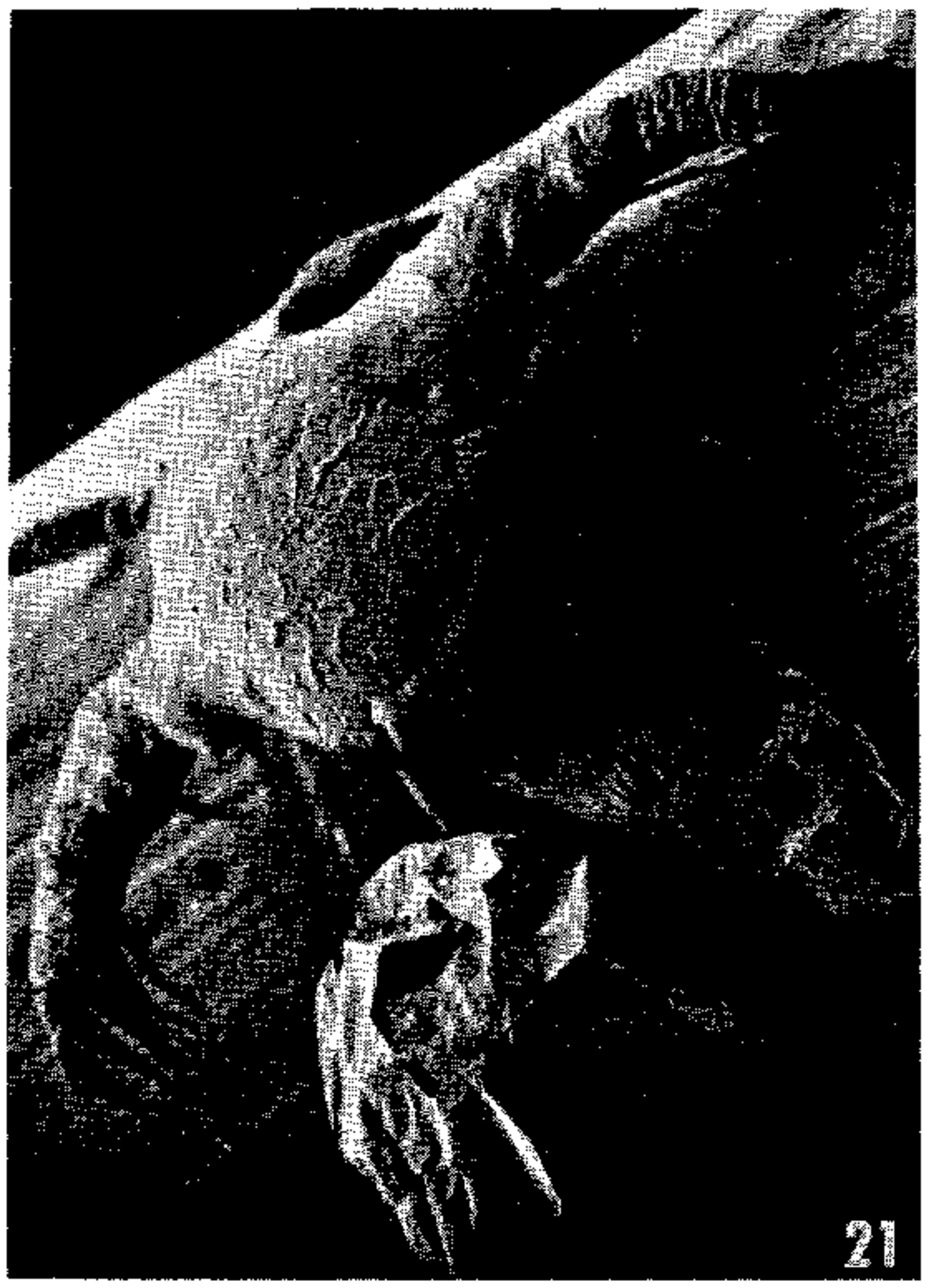
Fig.18. After its release from the cyst, the nauplius remains encased in the hatching membrane (HM). Note that the inner cuticular membrane (ICM) stays with the encased nauplius (× 180).

Fig.19. The hatching membrane (HM) ruptures at the posterior end (× 166).

Fig.20. A nauplius free of its hatching membrane but still conforming to the pear-shaped configuration (× 204).

brane (Figs. 18--20) and subsequent freeing of the larva. Figs. 21 and 22 illustrate newly emerged larvae and cast hatching membranes. In both micrographs (Figs 21 and 22) the larvae exhibit prominent regions of modified epithelium, neck glands, believed to be osmoregulatory in nature (Hootman et al., 1972).

The use of SEM has enabled a thorough examination of the hatching process as well as documenting the bacterial contamination, as it persists from vacuum packed cyst through hatching and apparently becomes manifest if the cysts have not hatched after 22 h. This study is preliminary to a complete SEM study of larval development in *Artemia salina*.



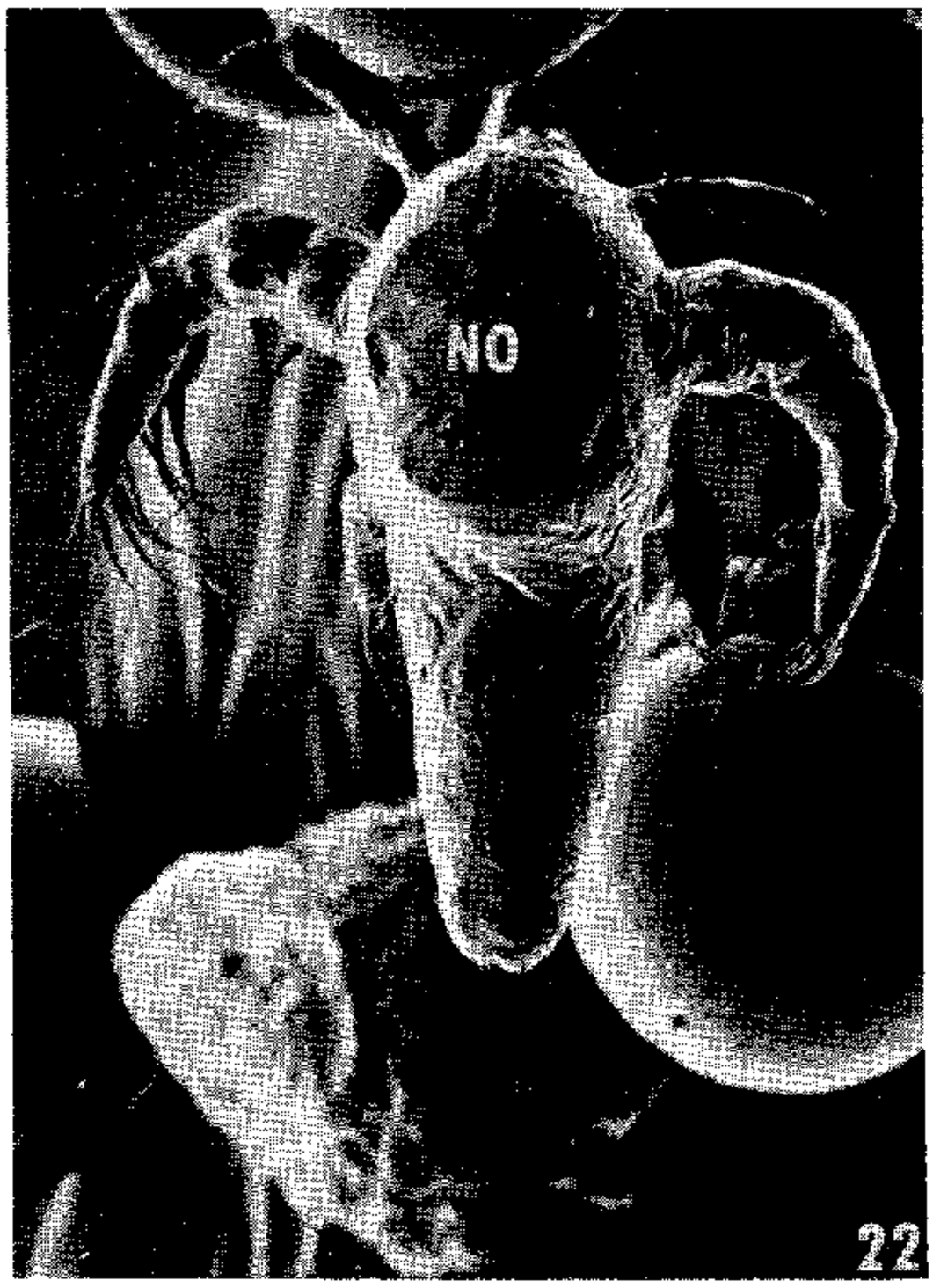


Fig.21. A nauplius with spread appendages beside an empty hatching membrane (HM) (× 152).

Fig. 22. A dorsal view of the nauplius showing the neck organ (NO) (× 140).

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